Final and Summary Report on Project

entitled

THE EFFECT OF MOLECULAR STRUCTURE ON CATALYSIS AND MOLECULAR BINDING

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April 16th 1962

Date

Work carried out under
Contract number DA 18-108-405-CML-265

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Final and Summary Report

MOLECULAR STRUCTURE AND CATALYTIC ACTIVITY

ABSTRACT

The present report constitutes the final report terminating Contract number DA-18-108-405-CML-265 covering the period November 1, 1958 to December 1, 1961. The objective of the investigations was the procurement of basic information relative to those functions which influence the activities of various molecular and ionic catalysts. The clear understanding of the mechanisms of action of molecular catalysis by chemical species in solution is of vital importance in the development of protective measures against chemical warfare agents. In these studies two different systems were investigated:

I. The hydrolytic behavior of sarin particularly under facilitating conditions.

II. The apparent bifunctional cleavage of the amide linkage in chloramphenicol.

The hydrolytic behavior of the methylfluorophosphonate ester was subjected to detail study because of its susceptibility to facilitation by certain functional groups and because of its continued importance as a chemical warfare agent. The present investigation has suggested that where molecular catalysis existed

for this system that there was substantial evidence of formation of an intermediate complex between the fluoroester and the catalytic species. These studies have also shown for the first time the correct chemical pathway followed during interaction of catechol and catechol derivatives. These latter results are believed to be of fundamental importance in understanding the so called "aging process" in producing irreversible inhibition by these agents.

The investigation of amide cleavage using chloramphenicol as the substrate indicates that dibasic acids facilitate the hydrolysis by means of bifunctional attack. In region of the pH profile where it has been shown that the reaction proceeds independent of hydrogen ion concentration, the rate of the reaction was shown to be directly dependent on the concentration of the half salts of the dibasic acids. In a series of dibasic aliphatic acids the effectiveness of the catalysts could be related to the sum of $pK_b + pK_{A_2}$ for the half salt which represents nucleophilic and electrophilic intensity of the bifunctional groups. Certain irregularities were noted but these appeared relatable to possible unfavorable steric factors.

Studies with cis and trans cyclohexane dicarboxylic acid were indicative of mediation of the amide cleavage by formation of molecular associations of the cis isomer with chloramphenicol.

Treatment of the data in the manner as previously done with the catechol-sarin system allowed estimation of the binding constant. It was interesting to note that the trans isomer exhibited no tendency to form complexes, indicating the marked specificity of the interaction.

INTRODUCTION

Clear understanding of mechanisms of action of molecular catalysis by chemical species in solution is of considerable importance in developing protective measures against chemical warfare agents. Detoxification of materials, protection of water supplies, decontamination of areas, and even treatment of exposed personnel can be more logically approached with greater understanding of this area. The work carried out under the contract was concerned with the procurement of basic information which would contribute to better understanding of these catalytic effects.

Several systems particularly susceptible to molecular catalysis were studied. These include:

1. Possible mediation of complexes in the nucleophilic attack on sarin.
2. Intramolecular participation in the hydrolysis of isopropyl o-hydroxyphenyl methyl phosphonate (SC).
3. Study of the reaction products of the hydrolysis of isopropyl o-hydroxy-3 nitropheyl methyl phosphonate.
4. Amide cleavage in chloramphenicol facilitated by bifunctional attack of dibasic carboxylic acids.

I. Possible Mediation of Complexes in the Nucleophilic Attack On Sarin.

It has previously been shown by Larsson (1) in Sweden and by Epstein (2) in this country that sarin reacts with such reagents as catechols and hydrogen peroxide at much higher rates than would ordinarily be expected from the basicity of these compounds. This rate enhancement was attributed qualitatively to the formation of intermediate complexes between Sarin and the catalytic reagents.

As reported in our previous communication (3) our treatment of the reaction of sarin with catechols, catechol Mannich bases and hypochlorite ion involved the assumption of the following mechanism:

$$S + C \underset{}{\overset{K}{\rightleftharpoons}} SC \xrightarrow{k_2} \text{Products.} \qquad (1)$$

where S represents sarin, C the catecholate ion or other reactive species, K the equilibrium constant and k_2 the rate constant for the breakdown of the intermediate complex. The mathematical formulation of this mechanism leads to the following rate law:

$$1/k_1' = 1/k_2K[C_0] + 1/k_2 \qquad (2)$$

where k_1' is the observed pseudo-first order rate constant when $[C]$ is in excess of $[S]$ and the subscript zero refers to the initial concentration.

From plots of $1/k_1'$ as a function of $1/C_0$ it is possible to evaluate the equilibrium constant, K, and the specific rate constant for the degradation of the intermediate complex. Figure 1,

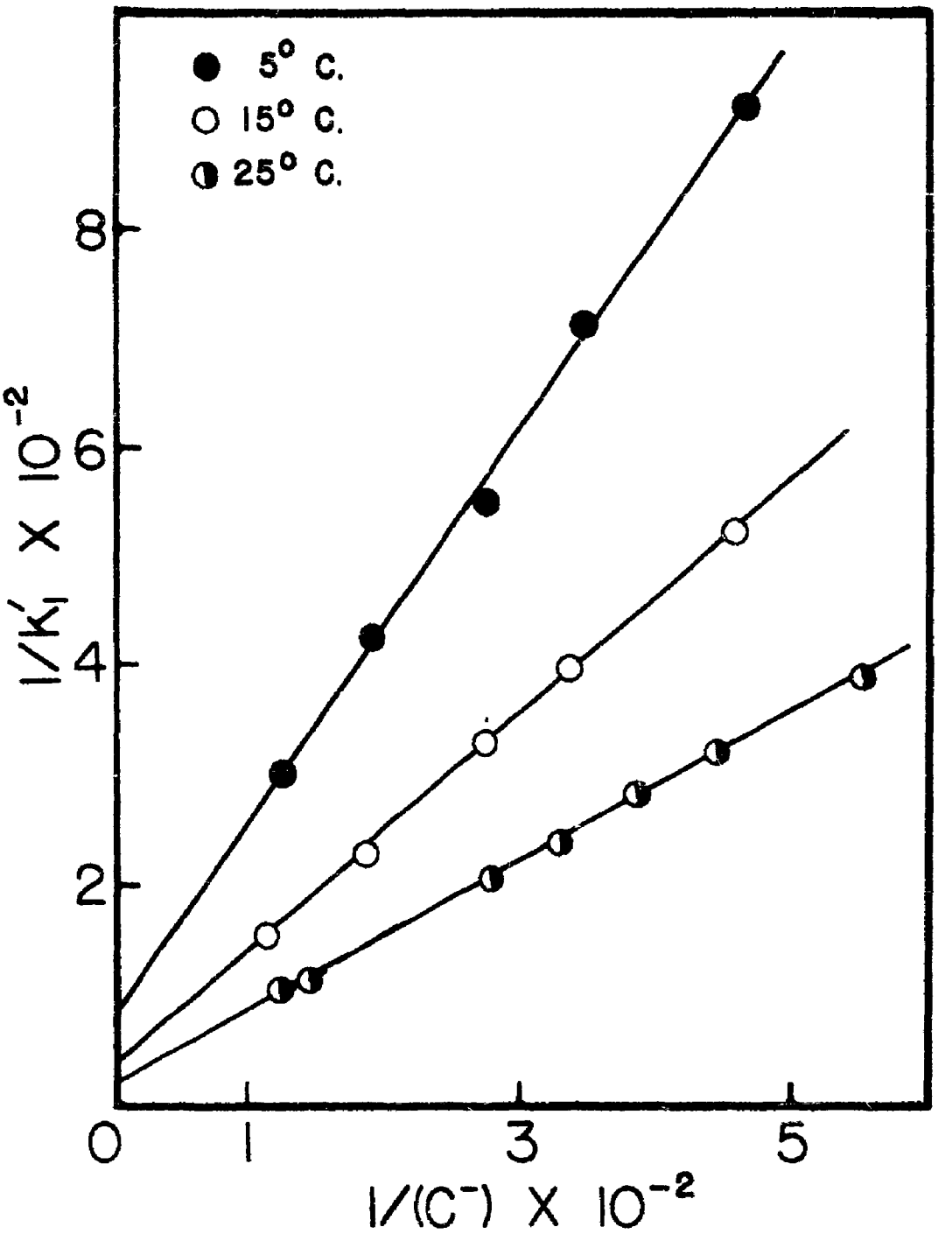


Figure 1

RECIPROCAL PLOTS FOR REACTION
OF SARIN WITH CUMA

representing the results obtained from the interaction of sarin with CDMA (3,6-Bis(dimethylaminomethyl)) catechol dihydrochloride at 5, 15 and 25°C. typifies the results obtained in these studies.

It is evident from the non-zero intercept that the reaction does proceed through an intermediate complex. While there is probably a relatively large error in the extrapolation of these lines to infinite dilution, there appears to be no doubt that the intercepts obtained are significantly different from zero.

Table I is a tabulation of the data obtained from the above type plot for a series of catechol derivatives. Within each group of compounds, i.e. catechols and Mannich bases there appears to be a qualitative relationship between k_2 and basicity of the ion. This is to be expected since the primary criterion for nucleophilic attack on an organophosphate has been shown to be the basicity of the nucleophile (4). It is evident from an analysis of the data that the rate of the degradation is not a direct function of the binding strength of the reactants as evidenced by the relationship of K, the equilibrium constant, and k_2. This would indicate that although associations may form, steric consideration are also of great importance. This situation may be treated as a competitive equilibrium as follows:

$$\begin{aligned} S + C^- &\underset{}{\overset{K_1}{\rightleftharpoons}} [SC^-]_1 \xrightarrow{k_2} \text{Products} \\ S + C^- &\overset{K_2}{\rightleftharpoons} [SC^-]_2 \end{aligned} \qquad (3)$$

TABLE I

Compound		25° C.			15° C.			5° C.		
Mannich Bases	pK_a	k_2[a]	K[b]	k_2K[c]	k_2[a]	K[b]	k_2K[c]	k_2[a]	K[b]	k_2K[c]
CDMA	6.35	6.3	23	1.42	3.0	31	0.92	1.3	41	0.53
CDEA	6.29	11.0	11	1.19	6.2	13	0.71	3.3	16	0.43
CDPM	6.39	11.0	12	1.30	5.5	13	0.81	2.7	16	0.52
DHNM	5.07	0.67	15	0.10	0.36	17	0.06	1.2[d]	13[d]	0.15[d]
Catechols										
Catechol	9.26	+	+	6.75						
3-Nitrocatechol	6.66	0.5	12	0.06						
4-Nitrocatechol	6.89	0.8	11	0.09						
Protocatechual-dehyde	7.23	2.0	9	0.18						
Protocatechuald-oxime	8.68	+	+	2.55						
2,3-Dihydroxy-naphthalene-6-sulfonate Na salt	8.30	+	+	0.82						
		15° C.			9° C.			1.1° C.		
HOCl	7.40	8.3	86	7.1	5.0	100	5.0	2.9	125	3.6

+ Intercept on reciprocal plot too low to determine k_2 and K

[a] x 10^2 sec.$^{-1}$ [b] liter mole^{-1} [c] liter mole^{-1} sec.$^{-1}$ [d] 35°C.

where K_1, k_2 and $[SC^-]_1$ have the same significance as described in equation (1) and K_2 being the equilibrium constant for the formation of the kinetically inactive complex SC^-_2. From mathematical treatment of this system it can be shown that equation (2) takes on the following form:

$$1/k_1' = 1/k_2K_1[C_0] + [K_1 + K_2]/k_2K_1 \qquad (4)$$

The only parameters which may be calculated from this equation are k_2K_1 and $(K_1 + K_2)$. The values for K listed in Table I may therefore actually be the sum of the two equilibrium constants

Similar studies were carried out on the p-nitrophenoxy analog of sarin, compound 1370 (5). This substance was utilized as its reaction is slower than that of sarin, therebye allowing higher concentration of the reactants. Figure 2 is representative of the results obtained in these experiments. The observed reactions appear to follow the mechanism formulated for the sarin degradation and Table II is a compilation of the results. The magnitude of k_2 is, in a general sense related directly to the basicity of the attacking species, but no such correlation can be made for the binding constant.

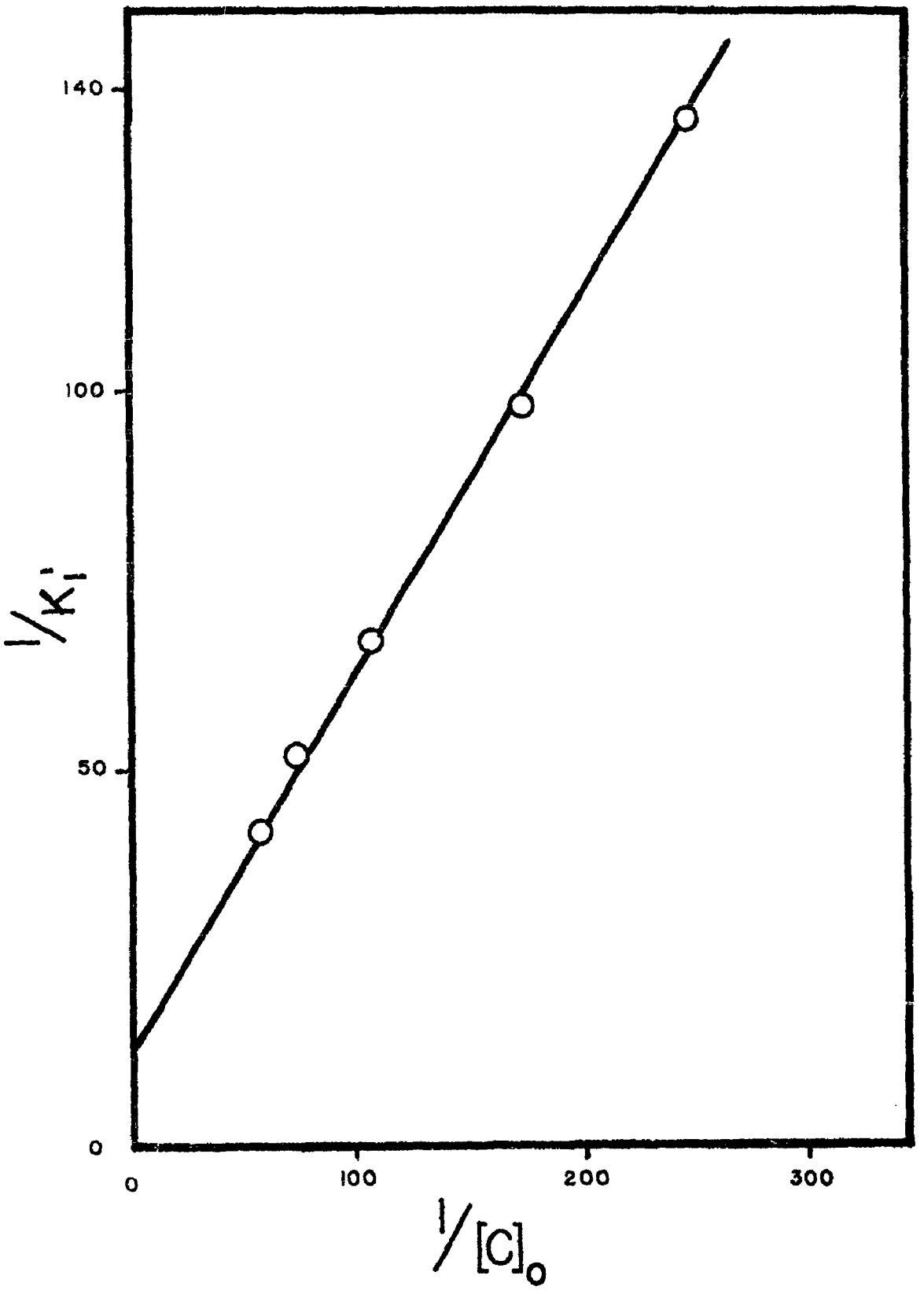


Figure 2

Reciprocal Plot for Reaction of 1370 with Methyl Gallate at 35° C.

TABLE II

Compound	Code name	pKa	$K(35^{o})$ (1/mole)	$k_2(35^{o})$ (min^{-1})	$k_2K(35^{o})$ (1/mole min)
Salicylaldoxime		8.93	8	0.78	6.24
1,2-Cyclohexanedione dioxime	DO	9.73	12	0.19	2.28
1,2,3-Cyclohexanetrione trioxime	TO	7.98	21	0.16	3.38
5-Methyl-1,2,3-cyclohexanetrione trioxime	MTO	7.99	8	0.39	3.12
5-Methyl-1,2,3-cyclohexanetrione-1,3-dioxime	MDO	8.60	10	0.84	8.4
Catechol		9.26	5	0.63	3.15
3,6-bis(dimethylaminomethyl) catechol dihydrochloride	CDMA	6.35	30	0.026	0.78
Pyrogallol		9.15	9	1.16	10.4
Gallic acid		8.55	35	0.15	5.3
Methyl gallate		7.90	31	0.064	1.90
Propyl gallate		8.05	18	0.108	1.94
Propylester of 2-diethylaminomethyl gallic acid dihydrochloride	GME	6.78	27	0.035	0.95
Propyl ester of 2,6-bis-(diethylaminomethyl)-gallic acid dihydrochloride	GDE	5.45	9	0.035	0.32

EXPERIMENTAL

Kinetic Procedure

The reaction assembly used in this work is shown in Figure 3. The reaction vessel consisted of a jacketed cell fitted with a polystyrene cover with inlets for the electrodes from the pH meter, nitrogen gas, and the ultraburet. There was also an opening for the removal of samples. Water at constant temperature was circulated through the cell jacket and the reaction mixture was stirred continously by means of a magnetic stirrer.

Exactly 100 ml. of a 0.1 molar potassium nitrate solution was transferred to the cell and an accurately weighed sample of catalyst was added. The solution was adjusted to the desired pH by the addition of sodium hydroxide solution and a sample removed for the blank analysis. The sarin or 1370 solution was then added, the timing begun, and samples were then withdrawn at appropriate time intervals.

Analytical Procedure.

The analysis of the sarin solutions was carried out by modification of the Schonemann method as previously reported (3).

The rate of 1370 hydrolysis was measured by the rate of appearance of p-nitrophenol. In the case where reactants imparted a color to the solution, the free p-nitrophenol was extracted with

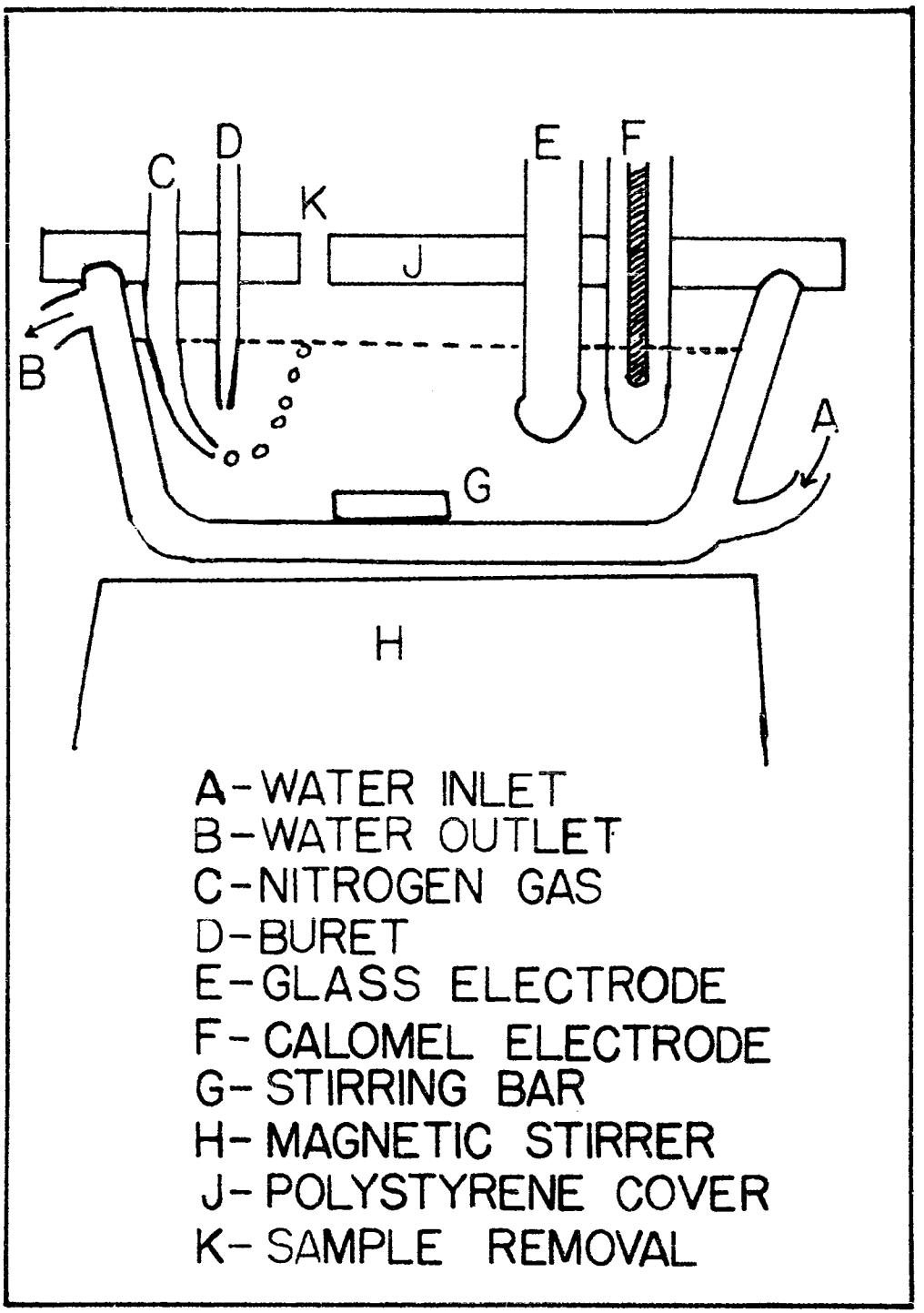
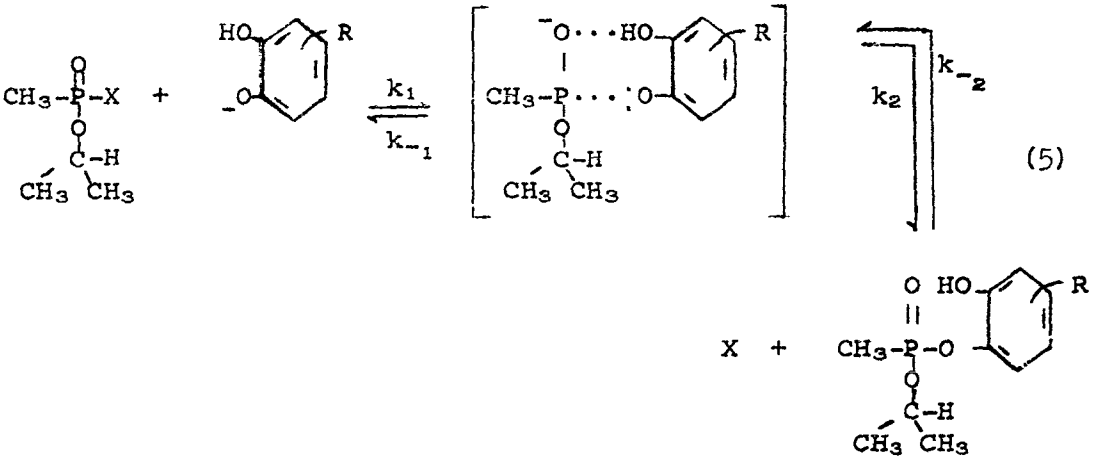


Figure 3 Reaction Assembly

xylene from an acidified solution and re-extracted into an aqueous alkaline solution.

II. Intramolecular participation in the hydrolysis of isopropyl o-hydroxyphenyl methyl phosphonate (SC).

If we consider the following generalized representation:



(5)

where X may be F or O-  $-NO_2$ and the R represents any substituted catecholate. If an equilibrium constant can be obtained for this reaction, then k_{-2} must be present. The initial concept and mathematical treatment of the data would indicate that if present, k_{-2} must be small. To establish this point, the reaction between 1370 and a number of compounds were studied. Since k_{-2}, if present would be small, an excess of p-nitrophenol was added to shift the equilibrium to the left.

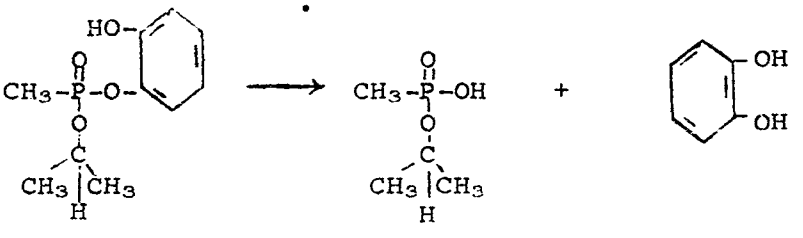
RESULTS

The results obtained with TO, propylgallate, 3 nitro catechol, and catechol indicated that no equilibrium existed, since no 1370

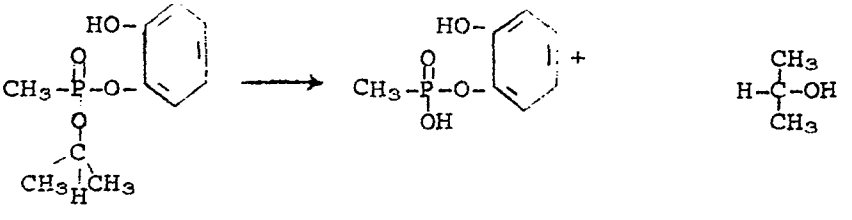
could be detected at the completion of the reaction. The interaction of 1370 with CDMA indicated that the final conentration of 1370 was independent of the amount of p-nitrophenol added. This behavior can not be rationalized on the basis of the proposed equilibrium, but on the basis of the interaction of another molecule of p-nitrophenol with the original addition product (5).

From the above findings it can be suggested that the initial products of the reaction undergo further degradation therebye preventing the establishment of an equilibrium.

The possible modes of degradation of the sarin-catechol addition product can be represented as follows:



(6)



(7)

From studies of the reaction at pH values varying from approximately 4 to 12, it was suggested in our earlier reports (5,6) that the reaction proceeded by pathway (7) above. This was

contrary to expectations based on the relative nucleophilicity of the leaving groups. It can be postulated that the presence of the ortho hydroxyl group has a stabilizing effect on the ortho hydroxy phenylene bond, resulting in the cleavage of the isopropoxy moiety.

Kinetic Dependency of the Reaction.

Isopropyl o-hydroxyphenyl methyl phosphonate was degraded over a wide pH range and the rate of the reaction was determined by following its dissappearance as a function of time. Although it was impossible to determine directly the rate of reaction at any pH in the absence of buffers because of the acidic nature of the reaction products, the corresponding rate was obtainable by extrapolation to zero buffer concentration of a series of results determined in the presence of buffers of varying concentrations. The pH profile thus obtained is shown in Figure 4, all measurements being made a 65°C. This profile can be represented as being the composite of three reactions:

$$SC + H_2O \xrightarrow{k_1} \text{Products}$$

$$SC^- + H_2O \xrightarrow{k_2} \text{"}$$

$$SC^- + OH^- \xrightarrow{k_3} \text{"}$$

where SC denotes isopropyl o-hydroxyphenyl methyl phosphonate and SC^- the dissociated compound. k_1, k_2, and k_3 represent the rate constants. From these three reactions it can be stated that :

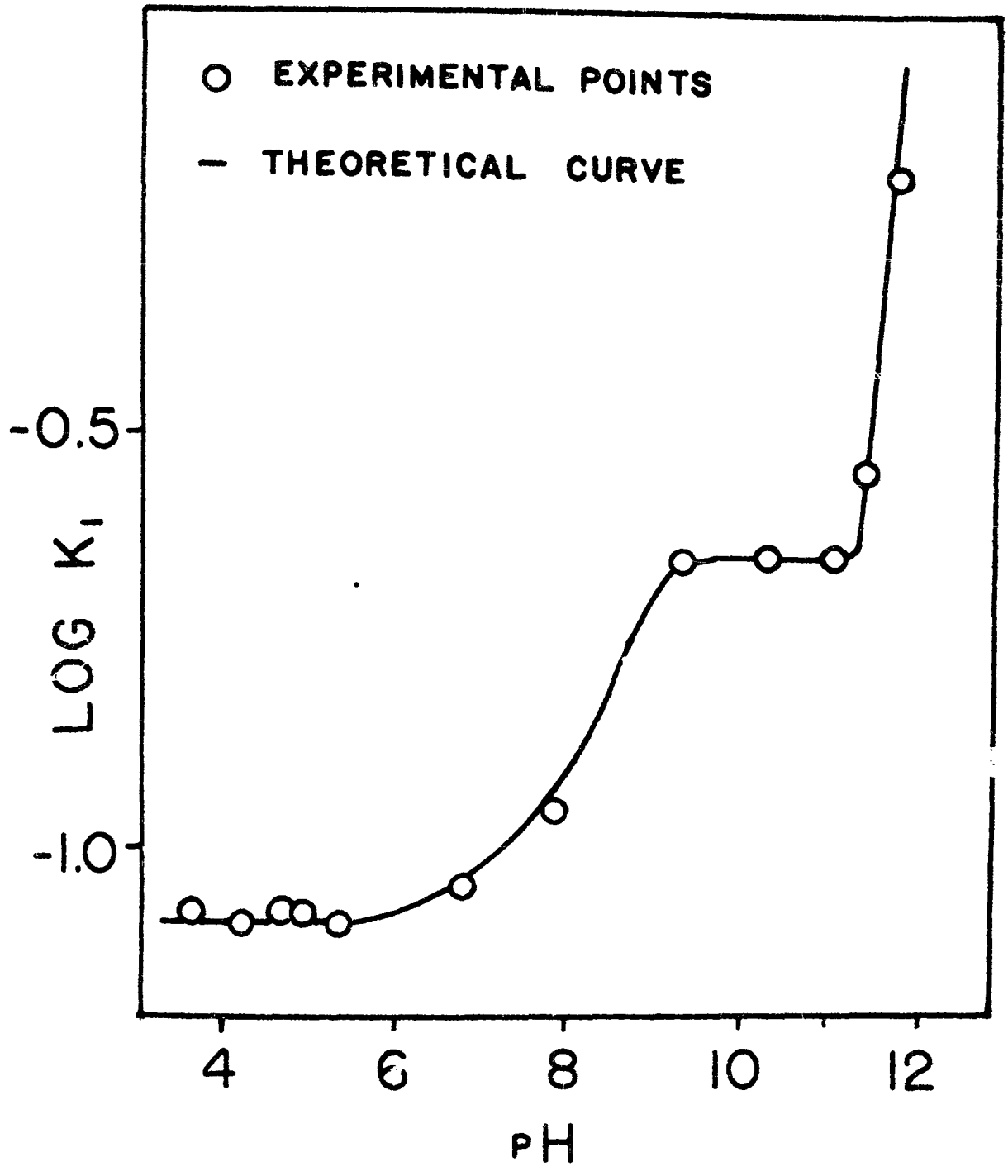


Figure 4

pH Profile of Isopropyl o-Hydroxyphenol Methyl Phosphonate Hydrolysis at 65°C.

$$\frac{-d(SC_t)}{dt} = k_1(SC) + k_2(SC^-) + k_3(SC^-)(OH^-)$$

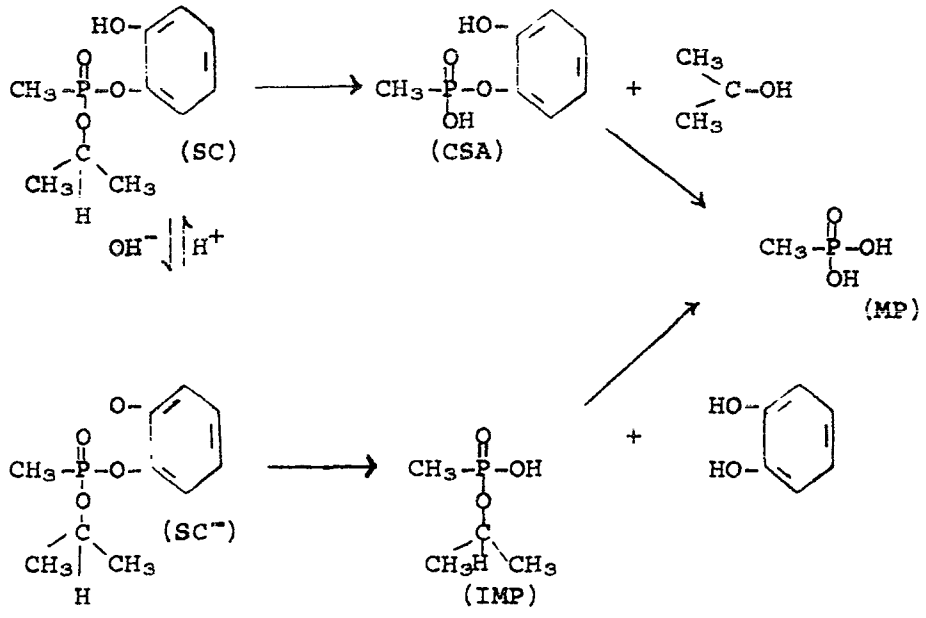
substitution for the SC and SC^- species in terms of their equilibrium concentrations yields the following:

$$k_{obs.} = k_1(H^+)/\left[K_a + (H^+)\right] + k_2K_a/\left[K_a + (H^+)\right]$$
$$k_3(OH^-)K_a/\left[K_a + (H^+)\right]$$

where $k_{obs.}$ is the observed first order disappearance of sarin-catechol. The solid line shown in Figure 3 was calculated from this equation.

Studies of similar systems using phenolate derivaties indicated the mechanism to be less complex (6) and the products of the degradation suggested the pathway of hydrolysis to be via the cleavage of the phenolate group rather than the isopropoxy group. This change in mechanism indicates the marked effect of the ortho hydroxy group on the reaction mechanism.

Current work in these laboratories indicates that the mechanism of the reaction changes at high pH, near 12. Paper chromatographic separation of the reaction products suggests that the ionized sarin-catechol might cleave at the catechol linkage rather than at the isopropoxy group. Under conditions of high pH it has been possible to identify products which were not previously reported by us. From this preliminary work the following sequence of reactions might be postulated:



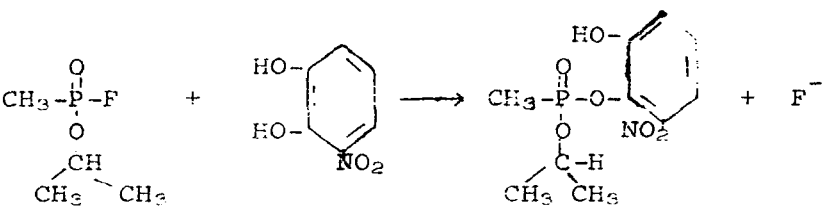
As noted, these studies are in a preliminary stage and as yet it is not possible to present kinetic data to completely support the above postulation. The aim of the present studies is to follow the rate of disappearance of SC and the rate of formation and nature of the degradation products as a function of pH.

III. Hydrolysis Products of Isopropyl o-hydroxy-3-nitrophenyl Methyl Phosphonate.

Due to the interest in the mechanism, of the sarin-catechol reaction, work was inaugurated on the 3-nitro derivative. This compound was chosen as the presence of the nitro group substantially lowers the pK_{a_1} and pK_{a_2} allowing the investigation of the reaction at lower pH.

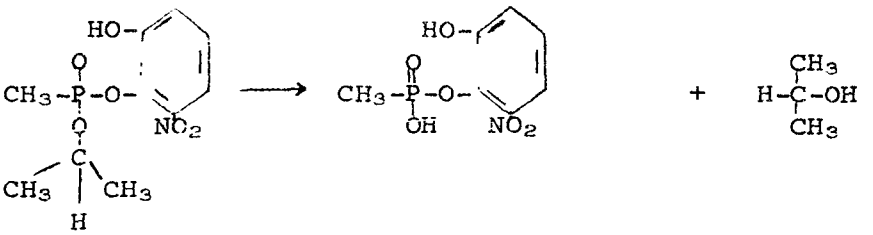
Sarin and 3-nitro catechol were allowed to react at pH 6.5

at 25°C. under an atmosphere of nitrogen as previously described for the sarin-catechol reaction. After four hours the reaction mixture was extracted with ether. Upon evaporation of the ether extract and separation of the residue on a silicic acid column it was possible to obtain the sarin-nitrocatechol (SNC) reaction product indicating the following reaction:

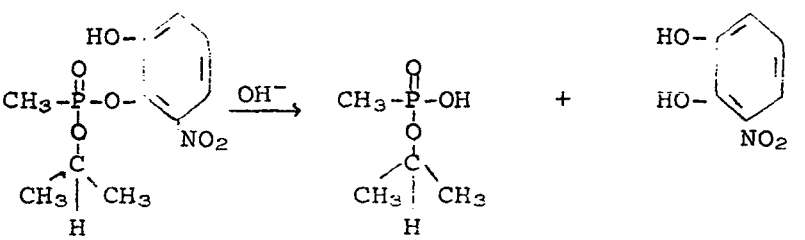


Acidification of the above reaction mixture and extraction with CCl_4 allowed separation of the products into two fractions. Sarin-nitro catechol and unreacted 3-nitro catechol being found in the carbon tetrachloride layer, and another compound having ultraviolet absorbance properies similar to SNC was found in the aqueous layer. This compound was extractable from the aqueous layer by ether. From the ethereal solution it was possible to obtain the compound in crystalline form. Based on UV, IR and elemental analysis it appears that the isolated material was the product formed by the isopropoxy cleavage of SNC. This belief was further supported by the fact that hydrolysis of the unknown substance under extremely acidic conditions yielded 3-nitrocatechol. Based on these results we may postulate that under neutral conditions sarin nitrocatechol cleaves in the manner previously reported

for sarin catechol according to the following equation:



The hydrolysis of sarin nitrocatechol at pH 12 yielded new products. Based on changes in the ultra-violet absorption spectra and detection of the formation of 3-nitro catechol by paper chromatographic techiques, it appears that at the high pH employed, that the mode of degradation changes to the cleavage of the 3-nitro catechol linkage as follows:



In efforts to clearly define the system, the kinetics of the over-all reaction are being studied and it is hoped that it will be possible to relate the observed kinetic results to the change in the degradative pathway.

This and the preceeding studies with the catechol derivative is believed to be of considerable importance in understanding the so-called "aging process" in producing irreversibility in agent inhibited enzymes. In the biochemical system dealkylation is also believed to take place and presents a pH profile similar to that

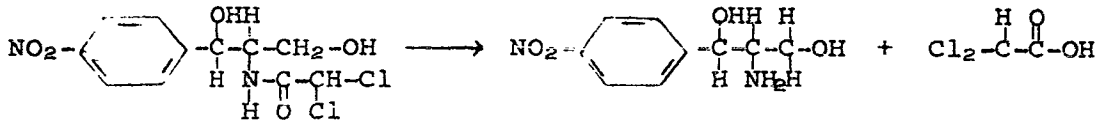
exhibited by the nitro catecholate ester.

IV. Amide Cleavage in Chloramphenicol Facilitated by Bifunctional Attack of Dibasic Carboxylic Acids.

It was found during the investigation of the kinetic behavior of chloramphenicol that the reaction was subject to general acid and general base catalysis (7). It was further noted that in the case of catalysis by a citrate system, that the partially neutralized forms of citric acid containing both acidic and basic groupings, exhibited greater catalytic activity than either the totally ionized species or the corresponding free acid. This interesting behavior led to the present investigation of the effects of bifunctional groups on the reaction rate and mechanism.

The study was designed to gain some insight into the variables influencing the efficacy of these catalysts as participants in the hydrolysis of chloramphenicol. The approximate second order catalytic constants for the half salts of oxalic, malonic succinic, glutaric, adipic, azelaic, malic, tartaric, maleic fumaric, phthalic, isophthalic, terephthalic, cis- and trans cyclohexane 1,2-dicarboxylic acid and ortho, meta and para hydroxybenzoic acids were determined at 96.4°C. The behavior of cis-cyclohexane 1,2-dicaroxylic acid was found to be of particular interest since the catalytic activity of its half salt exhibited a saturation effect characteristic of many enzyme reactions.

The degradation of chloramphenicol proceeds through the route of amide cleavage (8), and may be represented as follows:



It has been established as part of this study, that the end products of the reaction in the presence of bifunctional catalysts are the same as those reported above. This was verified by the isolation of the free amine from the reaction mixture and by analytically following the rate of formation of the amine and confirming that the rate of amine formation was equal to the rate of degradation of chloramphenicol.

Although the rate of the hydrolysis of the amide is essentially pH independent over the range 2-6 in buffer free systems (7), this is not the case in the presence of dicarboxylic acid buffer systems. The pH profile of the half lives obtained in the presence of tartrate buffers is shown in figure 5. Based on theoretical calculations it can be shown that the maxima exhibited by this curve corresponds with the pH at which the highest amount of the tartrate half salt can exist in solution. Similar relationships were found for the other bifunctional compounds investigated. The results in Table III show that relatively good agreement was noted between the calculated pH of maximum half salt concentration and the experimentally determined pH of optimim

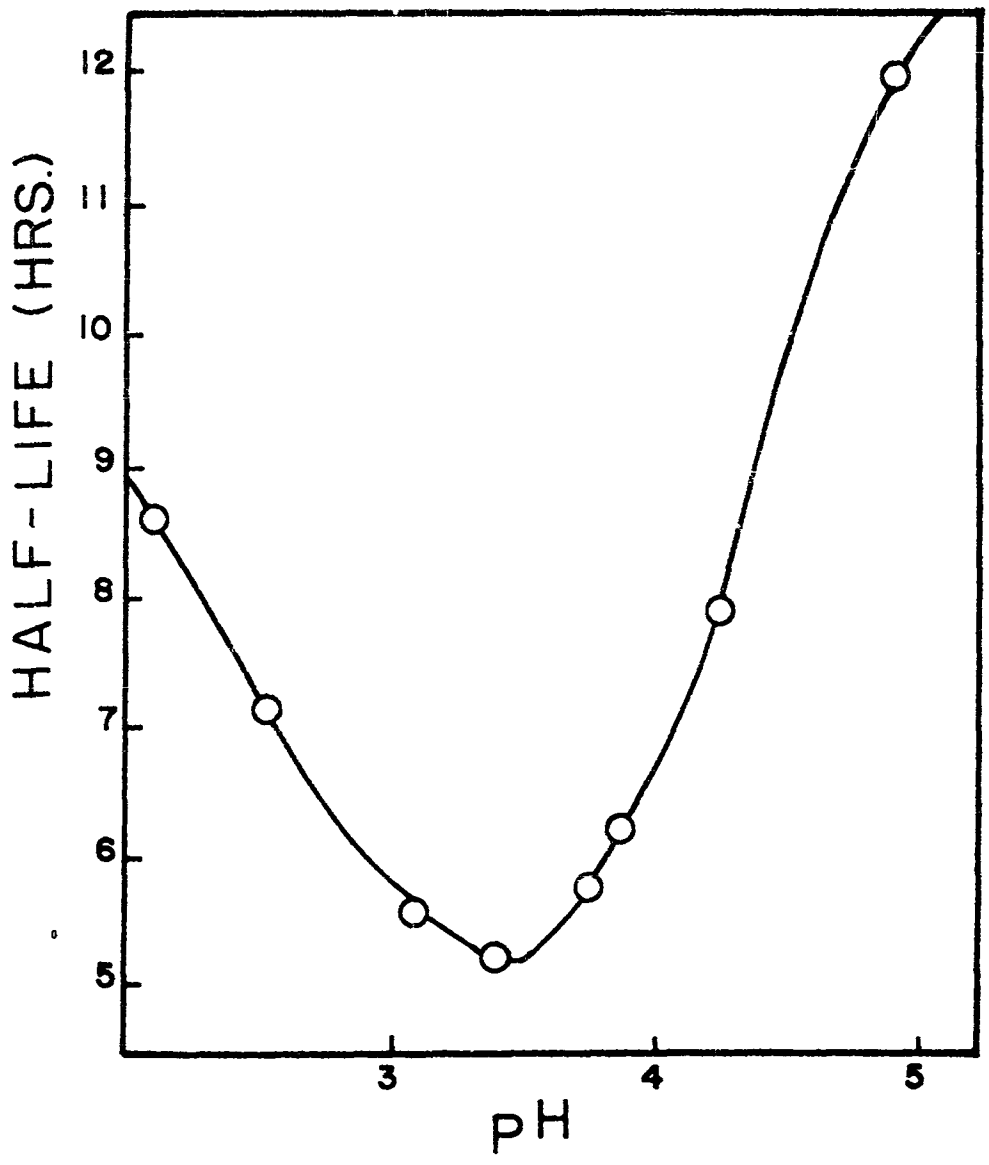


Figure 5

Plot showing the effect of pH on the catalytic activity, with respect to chloramphenicol hydrolysis, of a .1 M tartrate buffer. The pH's were determined at 25°C and the half-lives at 37.4 C.

TABLE III

Comparison of calculated pH for maximum half-salt concentration with observed pH of optimum catalysis

Buffer	Buffer Concentration	pH of Maximum Half-Salt Concentration[a]	pH of Optimum Catalysis[b]
Oxalate	0.4	3.5	3.5
Malonate	0.5	3.86	3.1
Succinate	0.5	4.6	3.7
Glutarate	0.5	4.78	4.25
Adipate	0.5	4.85	4.50
Malate	0.5	4.25	4.25
Tartrate	0.4	3.56	3.55
Maleate	0.5	3.86	3.00
Fumarate	0.5	3.75	3.65
Phthalate	0.5	4.25	4.25

a - calculated on the basis of literature value dissociation constants at 25°C.

b - pH determined at 25°C.

catalysis. The pH of maximum half salt concentration was calculated from literature dissociation constants determined at 25°C. for very dilute solutions. Since the kinetic studies were carried out at high temperature and high buffer concentrations, the pH shifts from the theoretical may be due to these effects.

In Table IV the approximate catalytic rate constants calculated from the kinetic runs made on a number of dicarboxylic acids are listed along with their "acid-base potentials". The latter values represent the sum of pK_b and pK_{a_2} for the half neutralized salts, and are a crude measure of the magnitude of the nucleophilic and electrophilic functions. It must be pointed out, however that any attempt to correlate "acid-base potential" with catalytic activity necessitates the assumption of an equal contribution of both the electrophilic and nucleophilic centers to the over-all reaction. While it has been established that both nucleophilic and electrophilic centers on the same molecule are necessary, the relative importance of each have not been evaluated.

Catalytic activities of cis and trans cyclohexane 1, 2 dicarboxylic acid half salts were studied in some detail because their cyclic structure constrained these molecules into fixed spatial configurations. Similar steric effects are present

TABLE IV

Half-Salt	Second Order Rate Constant[a,b,c,d]	"Acid-Base Potential"[e]
Oxalate	0.21	16.94
Malonate	0.14	16.83
Succinate	0.25	15.37
Glutarate	0.23	14.88
Adipate	0.35	14.85
Azelate	0.15	15.01
Terephthalate	0.06	15.31
Isophthalate	0.11	15.06
Phthalate	0.13	16.61
o-Hydroxy Benzoate	0.02	24.0
m-Hydroxy Benzoate	0.06	23.0
p-Hydroxy Benzoate	0.01	22.5
Maleate	0.06	18.07
Fumarate	0.35	15.52
Malate	0.29	15.65
Tartrate	0.46	15.20

a - Liters Moles^{-1} Hours $^{-1}$

b - Calculated assuming negligible catalytic effect from the undissociated and totally dissociated species.

c - Determined at 96.4°C

d - The effective half-salt concentrations have been calculated on the basis of literature value dissociation constants determined at 25°C. The fact that the kinetic investigations were carried out at 96.4°C must introduce some errors in the calculation of

CONT. TABLE IV

the listed second order rate constants. Because of the nature of these relationships at maximum half-salt concentration, however, it has been found that only those systems containing acids having K_{A_2} and K_{A_1} very close together would be seriously affected.

e - Calculated on the basis of literature value dissociation constants at 25°C.

in maleic and fumaric acids, however, the interpretation of these reactivities are clouded by the high degree of electronic conductivity provided by the double bond. The results not only confirmed the higher activity of the cis compound but suggested that the reaction was mediated by intermediate complex formation. Analysis of the data in a manner analogous to the previous treatment of the sarin-catechol type interaction bore out the supposition of complex formation. Figure 6 showing a plot of $1/k'$, the reciprocal of the observed pseudo first order rate constant, versus $1/C$, the reciprocal of the concentration of the half salt in the system is indicative of complex formation by virtue of the non-zero intercept. The equilibrium constant of the formation of the complex as calculated from Figure 5 was found to be 43.4 liters per mole. The study was repeated at 75°C. and the results are shown in Figure 7. It is of interest to note that the binding constant is considerably weaker than that found at 96.4°C.

Phase solubility studies were carried out as previously described (9) to permit independent determination of the extent of complex formation between the monoanion of cis cyclohexane 1, 2 dicarboxylic acid and chloramphenicol as a function of temperature. The non-linear dependency of the antibiotic solubility with respect to the half salt concentration, as shown in Figure 8 for several different temperatures, suggests the occurance of a rather involved interaction. Due to this complexity it was not

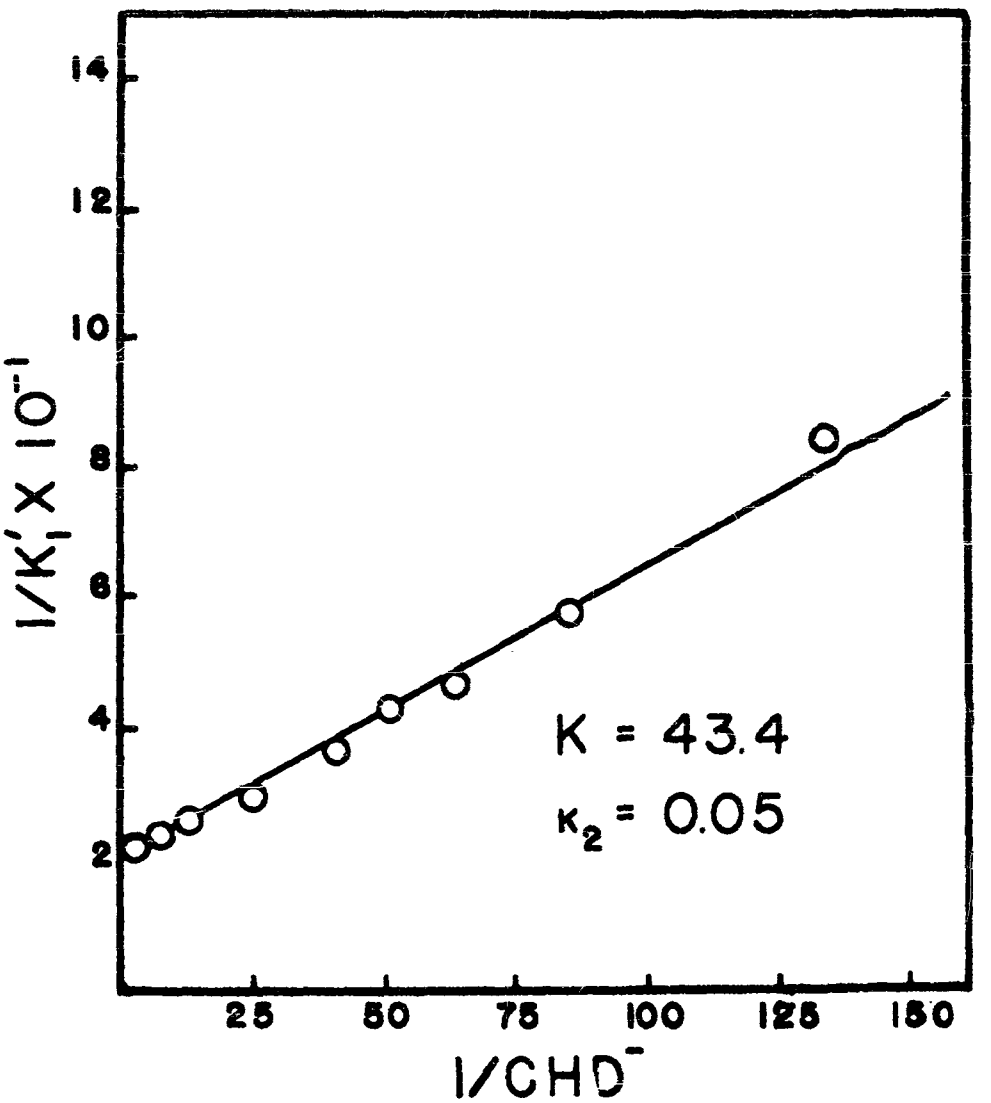


Figure 6

Reciprocal plot for the reaction of chloramphenicol with the half-neutralized salt of <u>cis</u> cyclohexane 1,2-dicarboxylic acid at 96.4°C.

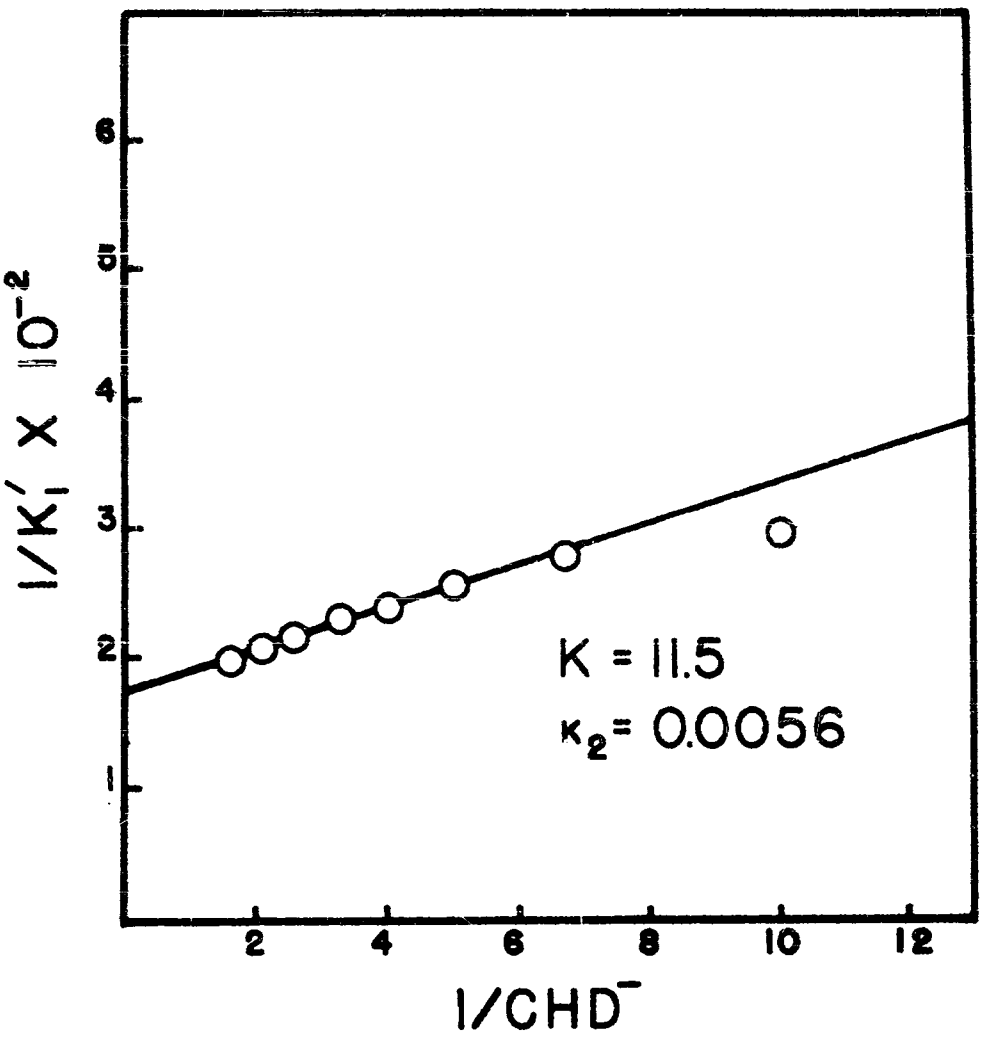


Figure 7

Reciprocal plot for the reaction of chloramphenicol with the half-neutralized salt of cis cyclohexane 1,2-dicarboxylic acid at 75°C.

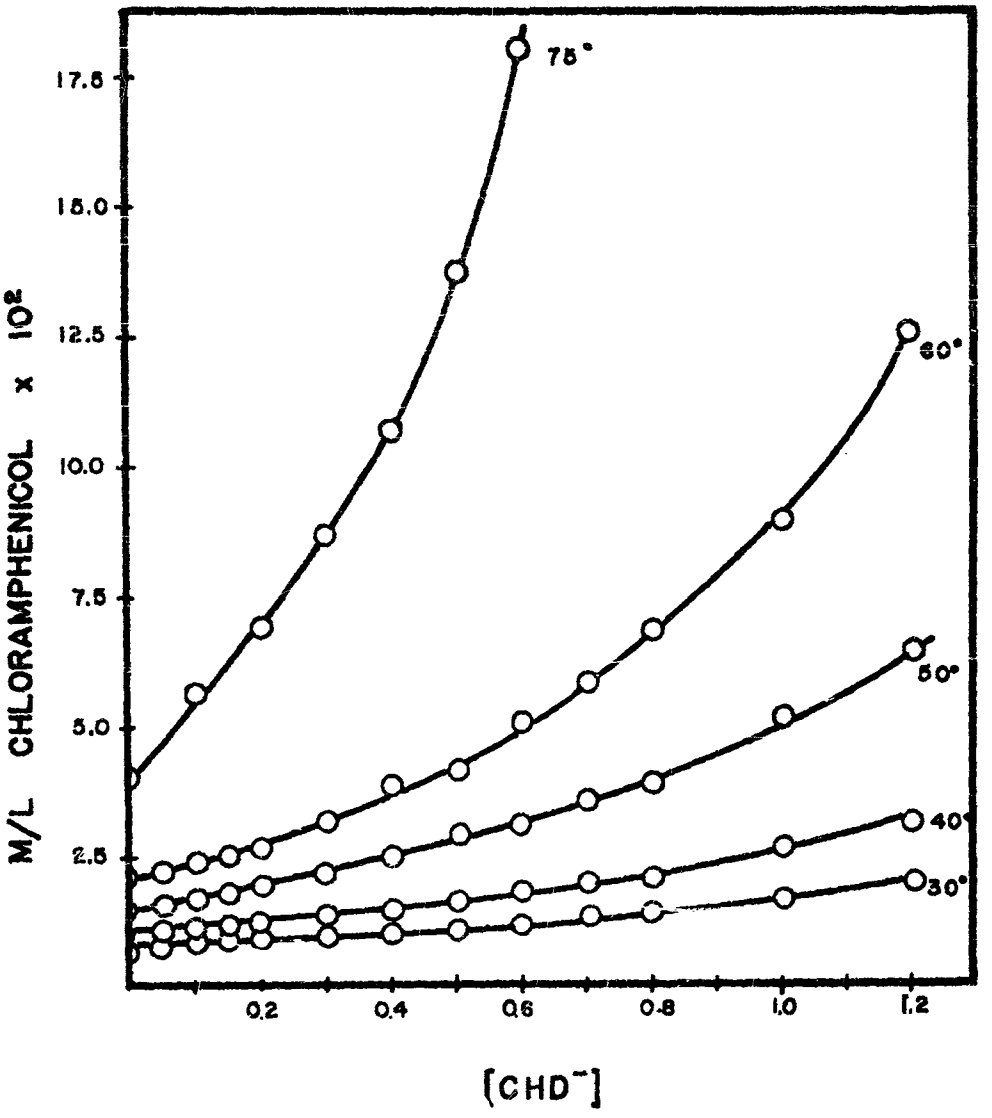


Figure 8

Phase diagrams showing the effect of temperature on the interaction between chloramphenicol and the monobasic salt of cis-cyclohexane dicarboxylic acid.

possible to calculate and compare interaction constants, but on the basis of the data at low half salt concentration, it can be assumed that the stoichiometry for the most part is a 1:1 ratio.

Phase solubility analysis as above carried out using trans cyclohexane 1,2 dicarboxylic acid indicated that the solubility of chloramphenicol was independent of the half salt concentration therebye suggesting that complex formation did not take place in this case.

Apparent Rate Enhancement as a Result of Intermediate Complex Formation

The half-salts discussed in the previous report do not appear to undergo significant complex formation with chloramphenicol and probably owe their activity to a random collision of the favorably oriented bifunctional molecule with the antibiotic substrate. The mono-anion of cis cyclohexane dicarboxylic acid, however, as been shown to undergo a rather strong, even if ill-defined, molecular association with chloramphenicol. Such an interaction may be expected to enhance the catalytic efficacy of the bifunctional molecule by increasing the effective catalyst concentration in the neighborhood of the substrate, thereby favoring the probability of the subsequent bifunctional attack.

In Table V are listed the apparent second order rate constants, as determined at 96.4°C, for monobasic cis and trans cyclohexane dicarboxylic acids as well as for the half-salts exhibiting the greatest catalysis in each of the previous series investigated. The catalytic rate constant for the half-neutralized species of trans cyclohexane dicarboxylic acid is shown to be of the same order of magnitude as the rate constants exhibited by the previously investigated half-salts. It is interesting to note, however, that the catalytic activity of the monobasic cis cyclohexane dicarboxylic acid, as reflected in the apparent second order rate constants, is approximately 5 times that of its nearest competitor, the half-salt of tartaric acid. This is particularly significant in view of the fact that the "acid-base potential" of the tartrate half-salt is about 20 times that of the equivalent cyclohexane dicarboxylic acid species. If one compares the catalytic activity of the cis cyclohexane dicarboxylic acid monoanion with that for the phthalate half-salt, a compound of similar "acid-base potential" and structural configuration, it is evident that the former is approximately 17 times more active a catalytic agent than is the phthalate half-salt. These results indicate the significant rate enhancement which may be achieved when a catalytic agent is constrained, in this case through complex formation, in the area of the substrate molecule.

TABLE V

Half-Salt	Second Order Rate Constant (1/mole hr.)	"Acid-Base Potential"[b]
Adipate	0.35[a,b,c]	14.85
Phthalate	0.13[a,b,c]	16.61
m-Hydroxybenzoate	0.06[a,b,c]	23.00
Fumarate	0.35[a,b,c]	15.52
Tartrate	0.46[a,b,c]	15.20
trans-Cyclohexane 1,2-Dicarboxylate	0.12[c]	15.73
cis-Cyclohexane 1,2-Dicarboxylate	2.7[c,d]	16.50

[a] Calculated on the basis of negligible catalytic effect from the undissociated and completely dissociated species.

[b] The effective half-salt concentrations and the "acid-base potentials" have been calculated on the basis of literature value dissociation constants determined at 25°C. The fact that the kinetic investigations were carried out at 96.4°C must introduce some errors in the calculation of the listed second order rate constants as well as the "acid-base potentials". Because of the nature of these relationships at maximum half-salt concentration, however, it has been found that only those systems containing acids having K_{A_1} and K_{A_2} very close together would be seriously affected.

[c] Determined at 96.4°C.

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